REMARKS

Upon entry of this amendment, claims 1, 5-21, 77-96 and 101-132 are pending in the instant application. Claims 1, 5-9, 11-18, 77-80, 82-84, 86, 88, 89, 91-93, and 95 have been amended, and claims 101-132 have been added. Support for the claim amendments presented herein is found throughout the specification and in the claims as originally filed. For example, support for the use of magnesium and manganese in the transcription reaction mixture is found at least in paragraph **[00120]**; support for the double-stranded oligonucleotide transcription template is found at least in paragraphs **[00128]** and **[00181]**; and support for the use of modified RNA polymerase that is able to incorporate any type of 2'-O-methyl nucleotide triphosphate (2'-OMe NTP) is found at least in paragraph **[00115]**. Support for new claims 101-132 is found throughout the specification, *e.g.*, in paragraphs **[0069]** through **[0077]** and **[0086]** through **[0088]**. Accordingly, the present amendments are fully supported, and no new matter has been added.

Claim Interpretation

With regard to the phrase "mutated polymerase", as used in claim 1, the Examiner has indicated that "there is no particular structure assigned in claim 1 to the mutated polymerase." (Office Action, page 2). The Examiner goes on to state that in fact the term "mutated" simply implies that the polymerase is changed relative to another polymerase sequence. Thus, the Examiner has interpreted claim 1, without any identification of the specific mutations involved, "broadly as reading on any polymerase, since any polymerase may be interpreted as 'mutated' relative to some other polymerase." (Office Action, page 2).

Applicants respectfully disagree with the Examiner's characterization and interpretation of the term "mutated polymerase" as broadly reading on any differing polymerases. However, merely to expedite the prosecution of the instant application, claim 1 has been amended to recite the use of a modified RNA polymerase comprising at least one mutated amino acid residue as compared to the amino acid sequence of the corresponding (i.e., the RNA polymerase from the same bacteriophage), unmodified RNA polymerase. Applicants submit that the skilled artisan would appreciate that a modified polymerase has a sequence and/or structure that differs from the unmodified version of that particular polymerase. As such, the term "modified RNA

polymerase", as recited by amended claim 1, should not be given an unreasonably broad interpretation so as to encompass any polymerase relative to any other polymerase.

Claim Rejections Under 35 U.S.C. § 102

Claims 1, 5, 9-11, 17, 19-22, 77 and 78 have been rejected under 35 U.S.C. § 102(b) as being anticipated by U.S. Patent No. 5,660,985 by Pieken et al. ("Pieken").

Claim 1 has been amended to recite the use of a modified RNA polymerase comprising at least one mutated amino acid residue as compared to the corresponding unmodified RNA polymerase, wherein the modified RNA polymerase exhibits an increased ability to incorporate a 2'-modified nucleotide triphosphate (NTP) as compared to the ability of the corresponding unmodified RNA polymerase to incorporate the NTP.

In contrast to the methods recited by the amended claims presented herein, Pieken does not teach the use of a modified RNA polymerase. Rather, the methods described by Pieken use a wild-type T7 RNA polymerase. Accordingly, the Pieken reference fails to disclose every element of the claimed methods. As such, the amended claims presented herein are novel over Pieken, and this rejection should be withdrawn.

Claim Rejections Under 35 U.S.C. § 103

Claims 6-8 have been rejected under 35 U.S.C. § 103(a) as being unpatentable over Pieken in view of Brieba et al., Biochemistry, vol. 39:919-923 (2000) ("Brieba"). Claims 12-16 have been rejected under 35 U.S.C. § 103(a) as being unpatentable over Pieken in view of U.S. Patent No. 6,107,037 by Sousa et al. ("Sousa"). Claim 18 has been rejected under 35 U.S.C. § 103(a) as being unpatentable over Pieken in view of Milligan et al., Methods Enzymol., vol. 180: 51-62 (1989) ("Milligan"). Claims 78-96 have been rejected under 35 U.S.C. § 103(a) as being unpatentable over Pieken in view of Sousa and in further view of Milligan.

Applicants traverse these rejections on the grounds that the Examiner has failed to establish a prima facie case of obviousness. A *prima facie* case of obviousness requires that "either the references must expressly or impliedly suggest the claimed invention or the examiner must present a convincing line of reasoning as to why the artisan would have found the claimed invention to have been obvious in light of the teachings of the references." *See* MPEP 706.02(j)

citing *Ex parte Clapp*, 227 USPQ 972, 973 (Bd. Pat. App. & Inter. 1985). Knowledge of the disclosure provided by the instant application must be put side when determining whether the claimed invention would have been obvious. *See* MPEP 2142.

To support the conclusion that the claimed invention is directed to obvious subject matter, the Examiner has cited and combined four references. The Pieken, Brieba, Sousa and Milligan references do not teach methods for identifying aptamers or transcribing oligonucleotides that comprise a 2'-OMe modified nucleotide using a transcription reaction mixture that includes a modified RNA polymerase that is capable of incorporating any type of 2'-OMe nucleotide triphosphate (*i.e.*, 2'-OMe ATP, 2'-OMe GTP, 2'-OMe CTP, 2'-OMe TTP and 2'-OMe UTP), wherein the mixture also includes magnesium ions, manganese ions and one or more double-stranded oligonucleotide transcription templates. In contrast to the methods of the claimed invention, the cited references do not teach the use of a modified RNA polymerase that is able to incorporate any type of 2'-OMe NTP at any position within an oligonucleotide transcript other than the 5' terminal nucleotide.

In fact, no combination of these references produces methods that use such a modified RNA polymerase in conjunction with a transcription reaction mixture that includes both magnesium and manganese ions, along with one or more double-stranded oligonucleotide transcription templates. The Pieken reference describes the use of wild-type T7 RNA polymerases and does not disclose any transcription reaction mixtures that include both magnesium and manganese ions. The Pieken reference also explicitly acknowledges that the wild-type T7 RNA polymerase does not recognize bulkier 2'-substituted NTPs, such as 2'-O-methyl. (*See* Pieken, col. 8, lines 26-29). Brieba describes the ability of a Y639/H784 double-mutant T7 RNA polymerase to incorporate NTPs with substituents capable of acting as hydrogen bond donors or acceptors (*e.g.*, 2'-OH and 2'-NH_2). However, Brieba is silent with regard to the use of any modified RNA polymerase to incorporate bulkier 2'-modified NTPs such as 2'-OMe NTPs, let alone a modified RNA polymerase that is capable of incorporating any type of 2'-OMe NTP. In addition, this reference does not disclose a transcription reaction mixture that includes both magnesium and manganese ions and a double-stranded oligonucleotide transcription template.

Sousa is silent with regard to the use of any modified RNA polymerase to incorporate bulkier 2'-modified NTPs such as 2'-OMe NTPs, let alone a modified RNA polymerase that is capable of incorporating any type of 2'-OMe NTP at any position within an oligonucleotide transcript other than the 5' terminal nucleotide. In addition, the Sousa reference does not teach the use of both magnesium and manganese ions in the transcription reaction mixture. Rather, the transcription conditions described in the Examples provided in Sousa use either magnesium or manganese, but never both in the same reaction condition. (*See* Sousa, col. 15, lines 44-46; col. 22, lines 4-50; and col. 29-30). In fact, Sousa teaches away from using manganese ions in the transcription reaction mixture by describing "a sharp reduction in overall [polymerase] activity with Mn++" that was seen over a wide range of Mn++ concentrations that were tested in an effort to identify an optimal Mn++ concentration. (*See* Sousa, col. 22, lines 34-50).

The addition of the Milligan reference fails to remedy the deficiencies in the teachings of Pieken, Brieba, Padilla, Sousa and Kujau, alone or in combination, as the Milligan reference does not disclose the use of a modified RNA polymerase that is able to incorporate any type of 2'-OMe NTP at any position within an oligonucleotide transcript other than the 5' terminal nucleotide or the use of a transcription reaction mixture that contains magnesium ions, manganese ions and a double-stranded oligonucleotide transcription template.

The mere fact that references can be combined or modified does not render the resultant combination obvious unless the results would have been predictable to one ordinary skill in the art. *See* MPEP §2143.01, citing *KSR International Co. v. Teleflex Inc.*, 550 U.S. ___, 82 USPQ2d 1385, 1396 (2007). Furthermore, a statement that modifications of the prior art to meet the claimed invention would have been "well within the ordinary skill of the art at the time the claimed invention was made" because the references relied upon teach that all aspects of the claimed invention were individually known in the art is not sufficient to establish a *prima facie* case of obviousness without some objective reason to combine the teachings of the references. *See* MPEP §2143.01, citing *Ex parte Levengood*, 28 USPQ2d 1300 (Bd. Pat. App. & Inter. 1993).

There is no objective reason provided by the Pieken, Brieba, Sousa, and Milligan references, alone or in combination, that would lead the skilled artisan to combine these references, nor is there any evidence that the resultant combination of these reference would have

been predictable. Moreover, these references fail to provide the skilled artisan with a reasonable expectation that the methods recited by the amended claims presented herein would successfully produce a mixture of transcripts that include at least one 2'-OMe modified nucleotide for use in identifying aptamers that bind to a target molecule.

None of the references cited by the Examiner describe the use of a modified polymerase to incorporate any type of 2'-OMe NTPs. In particular, the references cited fail to disclose the incorporation of 2'-OMe GTP in an oligonucleotide transcript. Those of ordinary skill in the art at the time of the instant invention would have recognized the difficulty in incorporating 2'-OMe GTP during oligonucleotide transcription. In an article published in September 2004 (Chelliserrykattil and Ellington, Nature Biotechnology, vol. 22(9): 1155-1160 (2004), copy enclosed herewith), Chelliserrykattil and Ellington admitted that all of the modified T7 RNA polymerase variants tested could not incorporate 2'-OMe GTP, and, thus, they could not produce an oligonucleotide transcript having 2'-OMe GTP incorporated therein. (*See e.g.*, Chelliserrykattil and Ellington, p. 1157, col. 2, lines 1-3 and Figure 2b, concluding that "none of the polymerases showed activity with 2'-O-methyl GTP").

The transcription reaction mixtures recited by the amended claims were carefully selected to produce a high yield of transcripts that contain at least one 2'-OMe modified nucleotide and are sufficiently long for effective and efficient use in the SELEX process. The transcription reaction mixture and conditions recited by the amended claims, particularly the use of both magnesium and manganese ions, allow the modified RNA polymerases to accept <u>any</u> of the 2'-OMe NTPs as substrates and incorporate these modified nucleotides into the transcript during both the initiation and elongation portions of transcription.

Furthermore, the claimed transcription reaction mixtures and conditions avoid problems regarding the accuracy with which sequence and population information is transmitted by the transcription and reverse-transcription steps in the SELEX process, which are commonly associated with the use of mutant polymerases, altered conditions and/or substituted nucleotides in transcription. These problems are often the result of high mutation rates (also known as "infidelity") during the transcription process, or the result of variation in transcription or reverse-transcription yields as a function of sequence or composition bias. Problems with bias and/or fidelity of transcription can render the SELEX process inefficient or even impossible.

The modified RNA polymerases, transcription reaction mixture and conditions recited by the amended claims overcome these problems. Aptamers identified using the methods recited by the amended claims presented herein have been shown to exhibit a high degree of fidelity, including perfect fidelity with no mutations, insertions or deletions. Likewise, composition bias in the aptamers made using the claimed methods has been shown to be very small and unlikely to alter the outcome of the SELEX process.

Accordingly, Applicants submit that there is no objective reason provided in any of the cited references, alone or in combination, that would lead the skilled artisan to arrive at the claimed invention. Moreover, there is no evidence that the results generated by combining these references would have been predictable, particularly in light of the inability of others in the field, *e.g.*, Chelliserrykattil and Ellington, even after the filing of the instant application, to identify and successfully use a modified polymerase that can incorporate all 2'-OMe NTPs, including 2'-OMe GTP to produce oligonucleotide transcripts. Thus, any suggestion that it would have been obvious to use the modified RNA polymerases, transcription reaction mixture and conditions in the methods recited by the amended claims presented herein is an improper application of hindsight based on Applicants' disclosure in the instant application. Thus, Applicants submit that the Examiner has failed to establish a *prima facie* case of obviousness and request that this rejection be withdrawn.

CONCLUSION

On the basis of the foregoing amendments, Applicants respectfully submit that the pending claims are in condition for allowance. If there are any questions regarding these amendments and remarks, the Examiner is encouraged to contact the undersigned at the telephone number provided below.

Respectfully submitted,

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